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(FILE 'HOME' ENTERED AT 14:21:47 ON 26 JUN 2003)

FILE 'REGISTRY' ENTERED AT 14:22:15 ON 26 JUN 2003
E CYTIDINE MONOPHOSPHATE-2-KETO-3-DEOXY-D-GLYCERO-D-GALACTO-NON

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SEA (SIALIC ACID OR NEU5AC)

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             4011   FILE TOXCENTER
             3044   FILE USPATFULL
               61   FILE USPAT2
                7   FILE VETB
               13   FILE VETU
              757   FILE WPIDS
              757   FILE WPINDEX
L1               QUE (SIALIC ACID OR NEU5AC)
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     FILE 'CAPLUS, MEDLINE, BIOSIS, EMBASE, SCISEARCH, TOXCENTER, BIOTECHNO,
     CANCERLIT, PASCAL' ENTERED AT 14:27:18 ON 26 JUN 2003
L2           456 S L1 AND (NONOIC ACID OR KDN)
L3            45 S L2 AND (CMP-KDN)
L4            11 DUP REM L3 (34 DUPLICATES REMOVED)
L5             8 S L2 AND (SIALIC ACID PHOSPHATE SYNTHASE OR SAS)
L6             2 DUP REM L5 (6
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=> d l4 ibib ab 1-11

L4 ANSWER 1 OF 11 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 1
ACCESSION NUMBER: 2001:591127 CAPLUS
DOCUMENT NUMBER: 135:300433
TITLE: Molecular cloning of a unique CMP-**sialic acid** synthetase that effectively utilizes both deaminoneuraminic acid (**KDN**) and N-acetylneuraminic acid (**Neu5Ac**) as substrates
AUTHOR(S): Nakata, Daisuke; Munster, Anja-K.; Gerardy-Schahn, Rita; Aoki, Naohito; Matsuda, Tsukasa; Kitajima, Ken
CORPORATE SOURCE: Department of Applied Molecular Biosciences, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, 464-8601, Japan
SOURCE: Glycobiology (2001), 11(8), 685-692
CODEN: GLYCE3; ISSN: 0959-6658
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB 2-Keto-3-deoxy-D-glycero-D-galacto-nononic acid (**KDN**) is a **sialic acid** (Sia) that is ubiquitously expressed in vertebrates during normal development and tumorigenesis. Its expression is thought to be regulated by multiple biosynthetic steps catalyzed by several enzymes, including CMP-Sia synthetase. Using crude enzyme prepns., it was shown that mammalian CMP-Sia synthetases had very low activity to synthesize **CMP-KDN** from **KDN** and CTP, and the corresponding enzyme from rainbow trout testis had high activity to synthesize both **CMP-KDN** and CMP-N-acetylneuraminic acid (**Neu5Ac**). To demonstrate if the unique substrate specificity found in the crude trout enzyme is conveyed by a single enzyme, cDNA cloning of trout CMP-Sia synthetase was carried out by PCR-based strategy. The trout enzyme was shown to consist of 432 amino acids with two potential nuclear localization signals, and the cDNA sequence displayed 53.8% identity to that of the murine enzyme. Based on the Vmax/Km values, the recombinant trout enzyme had high activity toward both **KDN** and **Neu5Ac** (1.1 vs. 0.68 min-1). In contrast, the recombinant murine enzyme had 15 times lower activity toward **KDN** than **Neu5Ac** (0.23 vs. 3.5 min-1). Northern blot anal. suggested that several sizes of the mRNA are expressed in testis, ovary, and liver in a tissue-specific manner. These results indicate that at least one cloned enzyme has the ability to utilize both **KDN** and **Neu5Ac** as substrates efficiently and is useful for the prodn. of **CMP-KDN**.
REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 2 OF 11 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 2
ACCESSION NUMBER: 2001:914804 CAPLUS
DOCUMENT NUMBER: 136:364440
TITLE: Cloning and expression of human **sialic acid** pathway genes to generate CMP-**sialic acids** in insect cells
AUTHOR(S): Lawrence, Shawn M.; Huddleston, Kathleen A.; Tomiya, Noboru; Nguyen, Nam; Lee, Yuan C.; Vann, Willie F.; Coleman, Timothy A.; Betenbaugh, Michael J.
CORPORATE SOURCE: Department of Chemical Engineering, The Johns Hopkins University, Baltimore, MD, 21218, USA
SOURCE: Glycoconjugate Journal (2001), 18(3), 205-213
CODEN: GLJOEW; ISSN: 0282-0080
PUBLISHER: Kluwer Academic Publishers
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The addn. of **sialic acid** residues to glycoproteins can affect important protein properties including biol. activity and in vivo circulatory half-life. For sialylation to occur, the donor sugar nucleotide cytidine monophospho-**sialic acid** (CMP-SA) must be generated and enzymically transferred to an acceptor oligosaccharide. However, examn. of insect cells grown in serum-free medium revealed negligible native levels of the most common **sialic acid** nucleotide, CMP-N-acetylneuraminic acid (CMP-**Neu5Ac**). To increase substrate levels, the enzymes of the metabolic pathway for CMP-SA synthesis have been engineered into insect cells using the baculovirus expression system. In this study, a human CMP-**sialic acid** synthase cDNA was identified and found to encode a protein with 94% identity to the murine homolog. The human CMP-**sialic acid** synthase (Cmp-Sas) is ubiquitously expressed in human cells from multiple tissues. When expressed in insect cells using the baculovirus vector, the encoded protein is functional and localizes to the nucleus as in mammalian cells. In addn., co-expression of Cmp-Sas with the recently cloned **sialic acid** phosphate synthase with N-acetylmannosamine feeding yields intracellular CMP-**Neu5Ac** levels 30 times higher than those obsd. in unsupplemented CHO cells. The absence of any one of these three components abolishes CMP-**Neu5Ac** prodn. in vivo. However, when N-acetylmannosamine feeding is omitted, the sugar nucleotide form of deaminated **Neu5Ac**, CMP-2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (**CMP-KDN**), is produced instead, indicating that alternative **sialic acid** glycoforms may eventually be possible in insect cells. The human CMP-SAS enzyme is also capable of CMP-N-glycolylneuraminic acid (CMP-Neu5Gc) synthesis when provided with the proper substrate. Engineering the CMP-SA metabolic pathway may be beneficial in various cell lines in which CMP-**Neu5Ac** prodn. limits sialylation of glycoproteins or other glycans.

REFERENCE COUNT: 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 3 OF 11 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 3

ACCESSION NUMBER: 2001:374227 CAPLUS
DOCUMENT NUMBER: 135:118967
TITLE: Determination of nucleotides and sugar nucleotides involved in protein glycosylation by high-performance anion-exchange chromatography: Sugar nucleotide contents in cultured insect cells and mammalian cells
AUTHOR(S): Tomiya, Noboru; Ailor, Eric; Lawrence, Shawn M.; Betenbaugh, Michael J.; Lee, Yuan C.
CORPORATE SOURCE: Department of Biology, Johns Hopkins University, Baltimore, MD, 21218, USA
SOURCE: Analytical Biochemistry (2001), 293(1), 129-137
CODEN: ANBCA2; ISSN: 0003-2697
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB We have developed a simple and highly sensitive HPLC method for detn. of cellular levels of sugar nucleotides and related nucleotides in cultured cells. Sepn. of 9 sugar nucleotides (CMP-**Neu5Ac**, CMP-Neu5Gc, **CMP-KDN**, UDP-Gal, UDP-Glc, UDP-GalNAc, UDP-GlcNAc, GDP-Fuc, GDP-Man) and 12 nucleotides (AMP, ADP, ATP, CMP, CDP, CTP, GMP, GDP, GTP, UMP, UDP, and UTP) was examd. by reversed-phase HPLC and high-performance anion-exchange chromatog. (HPAEC). Although the reversed-phase HPLC, using an ion-pairing reagent, gave a good sepn. of the 12 nucleotides, it did not sep. sufficiently the sugar nucleotides for quantification. On the other hand, the HPAEC method gave an excellent and reproducible sepn. of all nucleotides and sugar nucleotides with high sensitivity and reproducibility. We applied the HPAEC method to det. the intracellular sugar nucleotide levels of cultured Spodoptera frugiperda (Sf9) and Trichoplusia ni (High Five, BTN-TN-5B1-4) insect cells, and

compared them with those in Chinese hamster ovary (CHO-K1) cells. Sf9 and High Five cells showed concns. of UDP-GlcNAc, UDP-Gal, UDP-Glc, GDP-Fuc, and GDP-Man equal to or higher than those in CHO cells. CMP-**Neu5Ac** was detected in CHO cells, but it was not detected in Sf9 and High Five cells. In conclusion, the newly developed HPAEC method could provide valuable information necessary for generating sialylated complex-type N-glycans in insect or other cells, either native or genetically manipulated. (c) 2001 Academic Press.

REFERENCE COUNT: 71 THERE ARE 71 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 4 OF 11 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1999:558541 CAPLUS
DOCUMENT NUMBER: 132:10152
TITLE: Studies on enzymes involved in the formation and cleavage of the **KDN** residues in a new class of glycoconjugates containing **KDN**-glycan chains
AUTHOR(S): Terada, Takaho
CORPORATE SOURCE: Genomic Science Center (GSC), The Institute of Physical and Chemical Research (RIKEN), Saitama, 351-0198, Japan
SOURCE: Trends in Glycoscience and Glycotechnology (1999), 11(59), 147-152
CODEN: TGGLEE; ISSN: 0915-7352
PUBLISHER: FCCA
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review with 44 refs. **KDN** (2-keto-3-deoxy-D-glycero-D-galacto-nononic acid) is a unique form of **sialic acid** in which the aminoacyl group at C-5 in N-acylneuraminic acid is substituted by a hydroxyl group. The topics discussed in this review include identification and characterization of CTP:CMP-3-deoxynonulosonate cytidylyltransferase (**CMP-KDN** synthetase) from testis, substrate specificity of **CMP-KDN** synthetase, identification and characterization of **CMP-KDN** :lactosylceramide .alpha.2.fwdarw.3 **KDN** transferase, and studies on the reaction mechanism of a novel sialidase KDNase.

REFERENCE COUNT: 44 THERE ARE 44 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 5 OF 11 PASCAL COPYRIGHT 2003 INIST-CNRS. ALL RIGHTS RESERVED.

ACCESSION NUMBER: 1998-0471689 PASCAL
COPYRIGHT NOTICE: Copyright .COPYRGT. 1998 INIST-CNRS. All rights reserved.
TITLE (IN ENGLISH): CHEMOENZYMATIC SYNTHESIS OF SIALYL OLIGOSACCHARIDES AND ANALOGUES INVOLVED IN THE RECOGNITION WITH LECTINS IN METASTIC PROCESS
TITLE (IN FRENCH): SYNTHESE CHIMIO-ENZYMATIQUE D'OLIGOSACCHARIDES SIALYSLES ET D'ANALOGUES IMPLIQUES DANS LA RECONNAISSANCE AVEC LES LECTINES DANS LES PHENOMENES DE METASTASES
AUTHOR: SOMME Valerie; AUGE Claudine (dir.)
CORPORATE SOURCE: Universite de Paris 11, Orsay, France (tutelle)
SOURCE: (1998-01), 182 refs.
222 p.
Dissertation Information: Universite de Paris 11. Orsay. FRA, Th. doct., 98PA112019
DOCUMENT TYPE: Dissertation
BIBLIOGRAPHIC LEVEL: Monographic
COUNTRY: France
LANGUAGE: French
SUMMARY LANGUAGE: French; English
AVAILABILITY: INIST-T 119016, T98PA112019 0000; RBCCN-914712101,

T98PA112019 0000

L4 ANSWER 6 OF 11 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 4
ACCESSION NUMBER: 1998:233765 CAPLUS
DOCUMENT NUMBER: 129:14149
TITLE: Synthesis of neoglycoconjugates containing deaminated neuraminic acid (**KDN**) using rat liver .alpha.2,6-sialyltransferase
AUTHOR(S): Angata, Takashi; Matsuda, Tsukasa; Kitajima, Ken
CORPORATE SOURCE: Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, Tokyo, 113, Japan
SOURCE: Glycobiology (1998), 8(3), 277-284
CODEN: GLYCE3; ISSN: 0959-6658
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB 2-Keto-3-deoxy-D-glycero-D-galacto-nononic acid (**KDN**) was introduced into asialotransferrin and N-acetyllactosamine (LacNAc) from **CMP-KDN** by using rat liver Gal.beta.1.fwdarw.4GlcNAc .alpha.2,6-sialyltransferase to form **KDN**-transferrin and **KDN**-LacNAc. These structures contain terminal **KDN** .alpha.2.fwdarw.6Gal-residues, a glycotope that has not yet been described in natural glycoconjugates. **KDN** was transferred to all four Gal residues in asialotransferrin by this enzyme. The incorporation efficiency of **KDN** from **CMP-KDN** into asialotransferrin was about half that of **Neu5Ac** from CMP-**Neu5Ac**, based on the Vmax/Km values for these donor substrates, 0.0527 min-1 and 0.119 min-1, resp. The **KDN**.alpha.2.fwdarw.6Gal linkage was resistant to exosialidase treatment, in contrast to the sensitivity of the **Neu5Ac**.alpha.2.fwdarw.6Gal linkage. Interestingly, Sambucus sieboldiana agglutinin (SSA) was shown to prefer **KDN**-transferrin to the corresponding **Neu5Ac**-transferrin, as estd. by slot-blot anal. The use of an .alpha.2,6-sialyltransferase to synthesize neoglycoproteins contg. **KDN** has not been previously reported. Their facile synthesis using **CMP-KDN** and sialyltransferases with different specificities offers new possibilities to study the function of neo-**KDN**-glycoconjugates, and to explore their use in glycotechnol.
REFERENCE COUNT: 51 THERE ARE 51 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 5
ACCESSION NUMBER: 1998:533886 CAPLUS
DOCUMENT NUMBER: 129:276143
TITLE: Sialyltransferase-catalyzed transfer of **KDN** onto oligosaccharides
AUTHOR(S): Lubineau, Andre; Somme, Valerie; Auge, Claudine
CORPORATE SOURCE: URA CNRS 462, Laboratoire de Chimie Organique Multifonctionnelle, Universite PARIS-SUD, Orsay, 91405, Fr.
SOURCE: Journal of Molecular Catalysis B: Enzymatic (1998), 5(1-4), 235-240
CODEN: JMCEF8; ISSN: 1381-1177
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
OTHER SOURCE(S): CASREACT 129:276143
AB Sialyltransferases catalyze transfer of N-acetylneuraminic, the most common **sialic acid**, from cytidine 5-monophospho-N-acetylneuraminic acid, onto oligosaccharide chains. 3-Deoxy-.beta.-d-glycero-d-galacto-2-nonulopyranosonic acid (**KDN**), the deaminated analog of N-acetylneuraminic acid, was converted into **CMP-KDN** by a chem. procedure involving CMP phosphoramidite.

KDN was then successfully transferred, from **CMP-KDN**, onto Gal.beta.1-3(2OAc)Gal.beta.OCH3, in porcine liver .alpha.(2-3) sialyltransferase-catalyzed reaction, allowing prepn. of **KDN**.alpha.2-3Gal.beta.1-3(2OAc)Gal.beta.OCH3 in 88% yield. **KDN**.alpha.2-6Gal.beta.1-4GlcNAc could be also prepd. using rat liver sialyltransferase.

REFERENCE COUNT: 13 THERE ARE 13 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 8 OF 11 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 6

ACCESSION NUMBER: 1996:197572 CAPLUS
DOCUMENT NUMBER: 124:310943
TITLE: Substrate specificity of rainbow trout testis CMP-3-deoxy-D-glycero-D-galacto-nonulosonic acid (**CMP-Kdn**) synthetase. Kinetic studies of the reaction of natural and synthetic analogs of nonulosonic acid catalyzed by **CMP-Kdn** synthetase
AUTHOR(S): Terada, Takaho; Kitajima, Ken; Inoue, Sadako; Koppert, Klaus; Brossmer, Reinhard; Inoue, Yasuo
CORPORATE SOURCE: Dep. Biophysics and Biochem., Univ. Tokyo, Tokyo, Japan
SOURCE: European Journal of Biochemistry (1996), 236(3), 852-5
CODEN: EJBCAI; ISSN: 0014-2956
PUBLISHER: Springer
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Kinetic data of the activation reaction of several synthetic 3-deoxy-D-glycero-D-galacto-nonulosonic acid (**Kdn**) and N-acetylneuraminic acid (**Neu5Ac**) analogs catalyzed by the rainbow trout testis **CMP-Kdn** synthetase were presented. This enzyme showed broad substrate specificity in terms of substitutions at C4 at C5 position of **Kdn** and **Neu5Ac**. In contrast, calf brain CMP-N-acylneuraminic acid synthetase had narrow substrate specificity, being active only on various N-acyl analogs of New5Ac and only slightly active on **Kdn** derivs. Usefulness of the trout testis enzyme for synthesis of various CMP-sialate analogs, which could be donor substrates for sialyltransferases, was demonstrated.

L4 ANSWER 9 OF 11 MEDLINE

ACCESSION NUMBER: 95210899 MEDLINE
DOCUMENT NUMBER: 95210899 PubMed ID: 7696852
TITLE: Identification, characterization, and developmental expression of a novel alpha 2-->8-**KDN**-transferase which terminates elongation of alpha 2-->8-linked oligo-polysialic acid chain synthesis in trout egg polysialoglycoproteins.
AUTHOR: Angata T; Kitazume S; Terada T; Kitajima K; Inoue S; Troy F A 2nd; Inoue Y
CORPORATE SOURCE: Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, Japan.
CONTRACT NUMBER: AI 09352 (NIAID)
SOURCE: GLYCOCONJUGATE JOURNAL, (1994 Oct) 11 (5) 493-9.
Journal code: 8603310. ISSN: 0282-0080.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199505
ENTRY DATE: Entered STN: 19950510
Last Updated on STN: 19980206
Entered Medline: 19950504

AB A novel glycosyltransferase which catalyses transfer of deaminated neuraminic acid, **KDN** (2-keto-3-deoxy-D-glycero-D-galacto-nononic

acid) from **CMP-KDN** to the non-reducing termini of oligo-polysialyl chains of polysialoglycoprotein (PSGP), was discovered in the ovary of rainbow trout (Oncorhynchus mykiss). The **KDN** -transferase activity was optimal at neutral pH, and stimulated 2 to 2.5-fold by 2-5 mM Mg2+ or Mn2+. Expression of **KDN**-transferase was developmentally regulated in parallel with expression of the alpha 2-->8-polysialyltransferase, which catalyses synthesis of the oligo-polysialyl chains in PSGP. Incorporation of the **KDN** residues into the oligo-polysialyl chains prevented their further elongation, resulting in 'capping' of the oligo-polysialyl chains. This is the first example of a glycosyltransferase that catalyses termination of alpha 2-->8-polysialylation in glycoproteins.

L4 ANSWER 10 OF 11 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 7
ACCESSION NUMBER: 1993:403573 CAPLUS
DOCUMENT NUMBER: 119:3573
TITLE: Synthesis of CMP-deaminoneuraminic acid (**CMP -KDN**) using the CTP:CMP-3-deoxynonulosonate cytidylyltransferase from rainbow trout testis. Identification and characterization of a **CMP -KDN** synthetase
AUTHOR(S): Terada, Takaho; Kitazume, Shinobu; Kitajima, Ken; Inoue, Sadako; Ito, Fumio; Troy, Frederic A.; Inoue, Yasuo
CORPORATE SOURCE: Fac. Sci., Univ. Tokyo, Tokyo, 113, Japan
SOURCE: Journal of Biological Chemistry (1993), 268(4), 2640-8 CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The sugar nucleotide, CMP-3-deoxy-D-glycero-D-galacto-2-nonulosonate (**CMP-KDN**) is expected to serve as a donor of **KDN** residues in the synthesis of **KDN**-contg. glycoconjugates. Here, the identification and characterization of CMP-3-deoxynonulosonate cytidylyltransferase (**CMP-KDN** synthetase) (I), a novel enzyme responsible for synthesis of **CMP-KDN** from **KDN** and CTP, is reported. I was partially purified from the testis of rainbow trout (Oncorhynchus mykiss), where **KDN** gangliosides were 1st discovered, and used to synthesize CMP-[14C] **KDN**, which was characterized by 1H NMR. The Vmax/Km studies showed that **KDN** was a preferred nonulosonic acid substrate compared to N-acetylneuraminic acid (**Neu5Ac**) or N-glycolylneuraminic acid (Neu5Gc) (4.4 .times. 10-3 min-1 for **KDN** vs. 2.3 and 1.8 .times. 10-3 min-1 for **Neu5Ac** and Neu5Gc, resp.). I activity was maximal at pH 9-10 and at 25.degree.. The presence of either Mg2+ or Mn2+ was essential for I activity. Mg2+ (25 mM) stimulated the formation of **CMP-KDN** by >10-fold, yet only stimulated the formation of CMP-**Neu5Ac** and CMP-Neu5Gc 4-fold, relative to 1 mM Mg2+. A kinetic study using mixed substrates showed that both I and CMP-**Neu5Ac** synthetase activities in the partially purified enzyme were due to the same active site of a single enzyme. In contrast, **Neu5Ac** and Neu5Gc were the preferred nonulosonic acid substrates for the calf brain CMP-**sialic acid** synthetase. Thus, mammalian CMP-**sialic acid** synthetases recognize similar, yet distinctively different, substrate specificity determinants. Thus, the trout testis enzyme was considered to synthesize activated sugar nucleotides required for synthesis of both (**KDN**)GM3 and (**Neu5Ac**)GM3. The expression of I was shown to be temporally correlated with development and to parallel the developmental expression of (**KDN**)GM3 in sperm.

L4 ANSWER 11 OF 11 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1993:511878 CAPLUS
DOCUMENT NUMBER: 119:111878
TITLE: CMP-3-deoxynonulosonate synthetase which catalyzes the

transfer of CMP to **KDN** from CTP. Partial purification and characterization of the enzyme from rainbow trout testis

AUTHOR(S): Terada, Takaho; Kitazume, Shinobu; Kitajima, Ken; Inoue, Sadako; Ito, Fumio; Troy, Frederic A.; Inoue, Yasuo

CORPORATE SOURCE: Fac. Sci., Univ. Tokyo, Tokyo, 113, Japan

SOURCE: Polysialic Acid (1993), 191-9. Editor(s): Roth, Juergen; Rutishauser, Urs; Troy, Frederick A., II. Birkhaeuser: Basel, Switz.
CODEN: 59FNAM

DOCUMENT TYPE: Conference

LANGUAGE: English

AB The sugar nucleotide, cytidine 5'-(3-deoxy-D-glycero-D-galacto-2-nonulosonic phosphate) (**CMP-KDN**) is expected to serve as a donor of **KDN** residues in the synthesis of **KDN** -contg. glycoconjugates. The identification and characterization of **CMP-KDN** synthetase, a novel enzyme responsible for synthesis of **CMP-KDN** from **KDN** and CTP, is reported. The enzyme was partially purified from the testis of rainbow trout (Oncorhynchus mykiss), where **KDN**-gangliosides were first discovered, and used to synthesize CMP-[14C]**KDN**, which was characterized by 1H NMR. Vmax/Km studies showed that **KDN** was a preferred nonulosonic acid substrate compared to **Neu5Ac** or Neu5Gc. In contrast, **Neu5Ac** and Neu5Gc were the preferred nonulosonic acid substrates for the calf brain CMP-**sialic acid** synthetase. The presence of either Mg2+ or Mn2+ is essential for **CMP-KDN** synthetase activity. Kinetic and substrate specificity studies also showed that the trout testis enzymes could synthesize activated sugar nucleotides required for synthesis of both (**KDN**)GM3 and (**Neu5Ac**)GM3. The expression of **CMP-KDN** synthetase was shown to be temporally correlated with development of sperm.

=> d l6 ibib ab 1-2

L6 ANSWER 1 OF 2 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.DUPLICATE 1
ACCESSION NUMBER: 2003215546 EMBASE
TITLE: Comparative study of carbohydrate chains released from the oviducal mucins of the two very closely related amphibian species Bombina bombina and Bombina variegata.
AUTHOR: Coppin A.; Florea D.; Maes E.; Cogalniceanu D.; Strecker G.
CORPORATE SOURCE: G. Strecker, U. de Glycobiol. Struct. et Fonct., UMR 8576 du CNRS, Univ. des Sci. et Technol. de Lille, 59655 Villeneuve d'Ascq cedex, France. gerard.strecker@univ-lille1.fr
SOURCE: Biochimie, (2003) 85/1-2 (53-64).
Refs: 21
ISSN: 0300-9084 CODEN: BICMBE
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
010 Obstetrics and Gynecology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The eggs of amphibians are surrounded by an extracellular matrix, termed jelly coat, which is mainly composed of hydrated mucin-type glycoproteins. These highly glycosylated molecules are synthesized by the oviduct and play an important role in the fertilization process. From a structural and chemical point of view, these oviducal mucins are very different from one species to another and they could be involved in the species-specificity of gamete interactions or could influence the parasite tropism. Bombina bombina and Bombina variegata are the two most closely related species within the genus, which hybridize readily in nature. Divergence occurred during geographic isolation estimated at 2-7 million years ago. The oviducal mucins of these species have been studied at the carbohydrate level, and the primary structures of 28 compounds have been established by NMR spectroscopy. The carbohydrate chains released from the oviducal mucins of the two species were similar and characterized by the common sequences GlcNAc(.beta.1-3)[Fuc(.alpha.1-4)]GlcNAc(.beta.1-6) and GlcNAc(.alpha.1-4)Gal(.beta.1-4)Gal(.beta.1-3) attached to GalNAc-ol (core 2). Nevertheless, some differences confirmed the strict species-specificity of amphibian oviducal carbohydrate chains observed previously. On the one hand, the presence of .beta.Gal 1,4-linked to .beta.GlcNAc in B. bombina, but not in B. variegata, can indicate that .beta.4GalT: .beta.GlcNAc and .beta.4GalT: .beta.Gal are two distinct glycosyltransferases. On the other hand, deaminoneuraminic acid (**Kdn**) is present in B. bombina, and N-glycolylneuraminic acid (NeuGc) in B. variegata. Although the enzymes involved in the biosynthesis of **Kdn** are not as well characterized, it can be suggested that at least one step of the biosynthetic pathway of NeuAc has been disrupted, leading the B. bombina oviducal NeuAc-9-synthase to use Man-6-P as a substrate, instead of ManNAc-6-P. .COPYRGT. 2003 Editions scientifiques et medicales Elsevier **SAS** and Societe francaise de biochimie et biologie moleculaire. All rights reserved.

L6 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 2
ACCESSION NUMBER: 2001:914804 CAPLUS
DOCUMENT NUMBER: 136:364440
TITLE: Cloning and expression of human **sialic acid** pathway genes to generate CMP-**sialic acids** in insect cells
AUTHOR(S): Lawrence, Shawn M.; Huddleston, Kathleen A.; Tomiya, Noboru; Nguyen, Nam; Lee, Yuan C.; Vann, Willie F.; Coleman, Timothy A.; Betenbaugh, Michael J.
CORPORATE SOURCE: Department of Chemical Engineering, The Johns Hopkins University, Baltimore, MD, 21218, USA

SOURCE: Glycoconjugate Journal (2001), 18(3), 205-213
CODEN: GLJOEW; ISSN: 0282-0080
PUBLISHER: Kluwer Academic Publishers
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The addn. of **sialic acid** residues to glycoproteins can affect important protein properties including biol. activity and in vivo circulatory half-life. For sialylation to occur, the donor sugar nucleotide cytidine monophospho-**sialic acid** (CMP-SA) must be generated and enzymically transferred to an acceptor oligosaccharide. However, examn. of insect cells grown in serum-free medium revealed negligible native levels of the most common **sialic acid** nucleotide, CMP-N-acetylneuraminic acid (CMP-**Neu5Ac**). To increase substrate levels, the enzymes of the metabolic pathway for CMP-SA synthesis have been engineered into insect cells using the baculovirus expression system. In this study, a human CMP-**sialic acid** synthase cDNA was identified and found to encode a protein with 94% identity to the murine homolog. The human CMP-**sialic acid** synthase (Cmp-**Sas**) is ubiquitously expressed in human cells from multiple tissues. When expressed in insect cells using the baculovirus vector, the encoded protein is functional and localizes to the nucleus as in mammalian cells. In addn., co-expression of Cmp-**Sas** with the recently cloned **sialic acid phosphate synthase** with N-acetylmannosamine feeding yields intracellular CMP-**Neu5Ac** levels 30 times higher than those obsd. in unsupplemented CHO cells. The absence of any one of these three components abolishes CMP-**Neu5Ac** prodn. in vivo. However, when N-acetylmannosamine feeding is omitted, the sugar nucleotide form of deaminated **Neu5Ac**, CMP-2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (CMP-**KDN**), is produced instead, indicating that alternative **sialic acid** glycoforms may eventually be possible in insect cells. The human CMP-**SAS** enzyme is also capable of CMP-N-glycolylneuraminic acid (CMP-Neu5Gc) synthesis when provided with the proper substrate. Engineering the CMP-SA metabolic pathway may be beneficial in various cell lines in which CMP-**Neu5Ac** prodn. limits sialylation of glycoproteins or other glycans.

REFERENCE COUNT: 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d l4 ibib ab 1-11

L4 ANSWER 1 OF 11 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 1
ACCESSION NUMBER: 2001:591127 CAPLUS
DOCUMENT NUMBER: 135:300433
TITLE: Molecular cloning of a unique CMP-**sialic acid** synthetase that effectively utilizes both deaminoneuraminic acid (**KDN**) and N-acetylneuraminic acid (**Neu5Ac**) as substrates
AUTHOR(S): Nakata, Daisuke; Munster, Anja-K.; Gerardy-Schahn, Rita; Aoki, Naohito; Matsuda, Tsukasa; Kitajima, Ken
CORPORATE SOURCE: Department of Applied Molecular Biosciences, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, 464-8601, Japan
SOURCE: Glycobiology (2001), 11(8), 685-692
CODEN: GLYCE3; ISSN: 0959-6658
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB 2-Keto-3-deoxy-D-glycero-D-galacto-nononic acid (**KDN**) is a **sialic acid** (Sia) that is ubiquitously expressed in vertebrates during normal development and tumorigenesis. Its expression

is thought to be regulated by multiple biosynthetic steps catalyzed by several enzymes, including CMP-Sia synthetase. Using crude enzyme prepns., it was shown that mammalian CMP-Sia synthetases had very low activity to synthesize **CMP-KDN** from **KDN** and CTP, and the corresponding enzyme from rainbow trout testis had high activity to synthesize both **CMP-KDN** and CMP-N-acetylneuraminic acid (**Neu5Ac**). To demonstrate if the unique substrate specificity found in the crude trout enzyme is conveyed by a single enzyme, cDNA cloning of trout CMP-Sia synthetase was carried out by PCR-based strategy. The trout enzyme was shown to consist of 432 amino acids with two potential nuclear localization signals, and the cDNA sequence displayed 53.8% identity to that of the murine enzyme. Based on the Vmax/Km values, the recombinant trout enzyme had high activity toward both **KDN** and **Neu5Ac** (1.1 vs. 0.68 min-1). In contrast, the recombinant murine enzyme had 15 times lower activity toward **KDN** than **Neu5Ac** (0.23 vs. 3.5 min-1). Northern blot anal. suggested that several sizes of the mRNA are expressed in testis, ovary, and liver in a tissue-specific manner. These results indicate that at least one cloned enzyme has the ability to utilize both **KDN** and **Neu5Ac** as substrates efficiently and is useful for the prodn. of **CMP-KDN**.

REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 2 OF 11 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 2

ACCESSION NUMBER: 2001:914804 CAPLUS
DOCUMENT NUMBER: 136:364440
TITLE: Cloning and expression of human **sialic acid** pathway genes to generate CMP-**sialic acids** in insect cells
AUTHOR(S): Lawrence, Shawn M.; Huddleston, Kathleen A.; Tomiya, Noboru; Nguyen, Nam; Lee, Yuan C.; Vann, Willie F.; Coleman, Timothy A.; Betenbaugh, Michael J.
CORPORATE SOURCE: Department of Chemical Engineering, The Johns Hopkins University, Baltimore, MD, 21218, USA
SOURCE: Glycoconjugate Journal (2001), 18(3), 205-213
CODEN: GLJOEW; ISSN: 0282-0080
PUBLISHER: Kluwer Academic Publishers
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The addn. of **sialic acid** residues to glycoproteins can affect important protein properties including biol. activity and in vivo circulatory half-life. For sialylation to occur, the donor sugar nucleotide cytidine monophospho-**sialic acid** (CMP-SA) must be generated and enzymically transferred to an acceptor oligosaccharide. However, examn. of insect cells grown in serum-free medium revealed negligible native levels of the most common **sialic acid** nucleotide, CMP-N-acetylneuraminic acid (CMP-**Neu5Ac**). To increase substrate levels, the enzymes of the metabolic pathway for CMP-SA synthesis have been engineered into insect cells using the baculovirus expression system. In this study, a human CMP-**sialic acid** synthase cDNA was identified and found to encode a protein with 94% identity to the murine homolog. The human CMP-**sialic acid** synthase (Cmp-Sas) is ubiquitously expressed in human cells from multiple tissues. When expressed in insect cells using the baculovirus vector, the encoded protein is functional and localizes to the nucleus as in mammalian cells. In addn., co-expression of Cmp-Sas with the recently cloned **sialic acid** phosphate synthase with N-acetylmannosamine feeding yields intracellular CMP-**Neu5Ac** levels 30 times higher than those obsd. in unsupplemented CHO cells. The absence of any one of these three components abolishes CMP-**Neu5Ac** prodn. in vivo. However, when N-acetylmannosamine feeding is omitted, the sugar nucleotide form of deaminated **Neu5Ac**, CMP-2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (**CMP-KDN**), is produced

instead, indicating that alternative **sialic acid** glycoforms may eventually be possible in insect cells. The human CMP-SAS enzyme is also capable of CMP-N-glycolylneuraminic acid (CMP-Neu5Gc) synthesis when provided with the proper substrate. Engineering the CMP-SA metabolic pathway may be beneficial in various cell lines in which CMP-**Neu5Ac** prodn. limits sialylation of glycoproteins or other glycans.

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=> d his

     (FILE 'HOME' ENTERED AT 12:53:27 ON 26 JUN 2003)

     FILE 'REGISTRY' ENTERED AT 12:53:35 ON 26 JUN 2003
               E SIALIC ACID PHOSPHATE SYNTHASE/CN
L1             1 S E4

     FILE 'CA, CAPLUS' ENTERED AT 12:54:27 ON 26 JUN 2003
L2             2 S L1 AND (PURIF? OR CHARACT? OR CLON?)
L3             1 DUP REM L2 (1 DUPLICATE REMOVED)

     INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI,
     BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA,
     CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB,
     DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 12:56:54 ON
     26 JUN 2003
               SEA (SIALIC ACID PHOSPHATE SYNTHASE)
               ---------
               1   FILE BIOSIS
               1   FILE BIOTECHNO
               1   FILE CAPLUS
               1   FILE EMBASE
               1   FILE ESBIOBASE
               1   FILE MEDLINE
               1   FILE SCISEARCH
               1   FILE USPATFULL
L4              QUE (SIALIC ACID PHOSPHATE SYNTHASE)
               ---------

     FILE 'BIOSIS, BIOTECHNO, CAPLUS, EMBASE, ESBIOBASE, MEDLINE, SCISEARCH,
     USPATFULL, ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI,
     BIOBUSINESS, BIOCOMMERCE, BIOTECHDS' ENTERED AT 12:59:08 ON 26 JUN 2003
L5             1 S L1
L6             8 S (SIALIC ACID PHOSPHATE SYNTHASE)
L7             2 DUP REM L6 (6 DUPLICATES REMOVED)
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=> d l3 ibib ab

L3 ANSWER 1 OF 1 CA COPYRIGHT 2003 ACS DUPLICATE 1

ACCESSION NUMBER: 137:17958 CA

TITLE: Expression of a functional Drosophila melanogaster N-acetylneuraminic acid (Neu5Ac) phosphate synthase gene: Evidence for endogenous sialic acid biosynthetic ability in insects

AUTHOR(S): Kim, Kildong; Lawrence, Shawn M.; Park, Jung; Pitts, Lee; Vann, Willie F.; Betenbaugh, Michael J.; Palter, Karen B.

CORPORATE SOURCE: Department of Biology, Temple University, Philadelphia, PA, 19122, USA

SOURCE: Glycobiology (2002), 12(2), 73-83
CODEN: GLYCE3; ISSN: 0959-6658

PUBLISHER: Oxford University Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB In this study, we report the first **cloning** and **characterization** of a N-acetylneuraminic acid phosphate synthase gene from Drosophila melanogaster, an insect in the protostome lineage. The gene is ubiquitously expressed at all stages of Drosophila development and in Schneider cells. Similar to the human homolog, the gene encodes an enzyme with dual substrate specificity that can use either N-acetylmannosamine 6-phosphate or mannose 6-phosphate to generate phosphorylated forms of both the sialic acids, N-acetylneuraminic acid and 2-keto-3-deoxy-D-glycero-D-galacto-nononic acid, resp., when expressed in either bacterial or baculoviral expression systems. The identification of a functional sialic acid synthase in Drosophila indicates that insects have the biosynthetic capability to produce sialic acids endogenously. Although sialylation is widely distributed in organisms of the deuterstome lineage, genetic evidence concerning the presence or absence of sialic acid metab. in organisms of the protostome lineage has been lacking. Homol. searches of the Drosophila genome identified putative orthologues of other genes required for sialylation of glycoconjugates.

REFERENCE COUNT: 66 THERE ARE 66 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d l7 ibib ab 1-2

L7 ANSWER 1 OF 2 USPATFULL
ACCESSION NUMBER: 2002:258816 USPATFULL
TITLE: Engineering intracellular sialylation pathways
INVENTOR(S): Betenbaugh, Michael J., Baltimore, MD, UNITED STATES
Lawrence, Shawn, Dobbs Ferry, NY, UNITED STATES
Lee, Yuan C., Timonium, MD, UNITED STATES
Coleman, Timothy A., Gaithersburg, MD, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002142386	A1	20021003
APPLICATION INFO.:	US 2001-930440	A1	20010816 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-227579P	20000825 (60)
	US 1999-169624P	19991208 (60)
	US 1999-122582P	19990302 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: HUMAN GENOME SCIENCES INC, 9410 KEY WEST AVENUE, ROCKVILLE, MD, 20850
NUMBER OF CLAIMS: 47
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 40 Drawing Page(s)
LINE COUNT: 4472
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Methods for manipulating carbohydrate processing pathways in cells of interest are provided. Methods are directed at manipulating multiple pathways involved with the sialylation reaction by using recombinant DNA technology and substrate feeding approaches to enable the production of sialylated glycoproteins in cells of interest. These carbohydrate engineering efforts encompass the implementation of new carbohydrate bioassays, the examination of a selection of insect cell lines and the use of bioinformatics to identify gene sequences for critical processing enzymes. The compositions comprise cells of interest producing sialylated glycoproteins. The methods and compositions are useful for heterologous expression of glycoproteins.

L7 ANSWER 2 OF 2 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
ACCESSION NUMBER: 2002:56015 BIOSIS
DOCUMENT NUMBER: PREV200200056015
TITLE: Cloning and expression of human sialic acid pathway genes to generate CMP-sialic acids in insect cells.
AUTHOR(S): Lawrence, Shawn M.; Huddleston, Kathleen A.; Tomiya, Noboru; Nguyen, Nam; Lee, Yuan C.; Vann, Willie F.; Coleman, Timothy A.; Betenbaugh, Michael J. (1)
CORPORATE SOURCE: (1) Department of Chemical Engineering, Johns Hopkins University, 3400 N. Charles St., Baltimore, MD, 21218: beten@jhu.edu USA
SOURCE: Glycoconjugate Journal, (March, 2001) Vol. 18, No. 3, pp. 205-213. print.
ISSN: 0282-0080.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The addition of sialic acid residues to glycoproteins can affect important protein properties including biological activity and in vivo circulatory half-life. For sialylation to occur, the donor sugar nucleotide cytidine monophospho-sialic acid (CMP-SA) must be generated and enzymatically transferred to an acceptor oligosaccharide. However, examination of insect cells grown in serum-free medium revealed negligible native levels of the

most common sialic acid nucleotide, CMP-N-acetylneuraminic acid (CMP-Neu5Ac). To increase substrate levels, the enzymes of the metabolic pathway for CMP-SA synthesis have been engineered into insect cells using the baculovirus expression system. In this study, a human CMP-sialic acid synthase cDNA was identified and found to encode a protein with 94% identity to the murine homologue. The human CMP-sialic acid synthase (Cmp-Sas) is ubiquitously expressed in human cells from multiple tissues. When expressed in insect cells using the baculovirus vector, the encoded protein is functional and localizes to the nucleus as in mammalian cells. In addition, co-expression of Cmp-Sas with the recently cloned **sialic acid phosphate synthase** with N-acetylmannosamine feeding yields intracellular CMP-Neu5Ac levels 30 times higher than those observed in unsupplemented CHO cells. The absence of any one of these three components abolishes CMP-Neu5Ac production in vivo. However, when N-acetylmannosamine feeding is omitted, the sugar nucleotide form of deaminated Neu5Ac, CMP-2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (CMP-KDN), is produced instead, indicating that alternative sialic acid glycoforms may eventually be possible in insect cells. The human CMP-SAS enzyme is also capable of CMP-N-glycolylneuraminic acid (CMP-Neu5Gc) synthesis when provided with the proper substrate. Engineering the CMP-SA metabolic pathway may be beneficial in various cell lines in which CMP-Neu5Ac production limits sialylation of glycoproteins or other glycans.

=> d l3 ibib ab

L3 ANSWER 1 OF 1 CA COPYRIGHT 2003 ACS DUPLICATE 1
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AUTHOR(S): Kim, Kildong; Lawrence, Shawn M.; Park, Jung; Pitts, Lee; Vann, Willie F.; Betenbaugh, Michael J.; Palter, Karen B.
CORPORATE SOURCE: Department of Biology, Temple University, Philadelphia, PA, 19122, USA
SOURCE: Glycobiology (2002), 12(2), 73-83
CODEN: GLYCE3; ISSN: 0959-6658
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In this study, we report the first **cloning** and **characterization** of a N-acetylneuraminic acid phosphate synthase gene from Drosophila melanogaster, an insect in the protostome lineage. The gene is ubiquitously expressed at all stages of Drosophila development and in Schneider cells. Similar to the human homolog, the gene encodes an enzyme with dual substrate specificity that can use either N-acetylmannosamine 6-phosphate or mannose 6-phosphate to generate phosphorylated forms of both the sialic acids, N-acetylneuraminic acid and 2-keto-3-deoxy-D-glycero-D-galacto-nononic acid, resp., when expressed in either bacterial or baculoviral expression systems. The identification of a functional sialic acid synthase in Drosophila indicates that insects have the biosynthetic capability to produce sialic acids endogenously. Although sialylation is widely distributed in organisms of the deuterstome lineage, genetic evidence concerning the presence or absence of sialic acid metab. in organisms of the protostome lineage has been lacking. Homol. searches of the Drosophila genome identified putative orthologues of other genes required for sialylation of glycoconjugates.
REFERENCE COUNT: 66 THERE ARE 66 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT